SUPPLEMENTARY DATA

Preclinical evaluation of miR-15/107 family members as multifactorial drug targets for Alzheimer's disease

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1) Supplementary Methods

2) Supplementary Figures and legends

3) Supplementary References

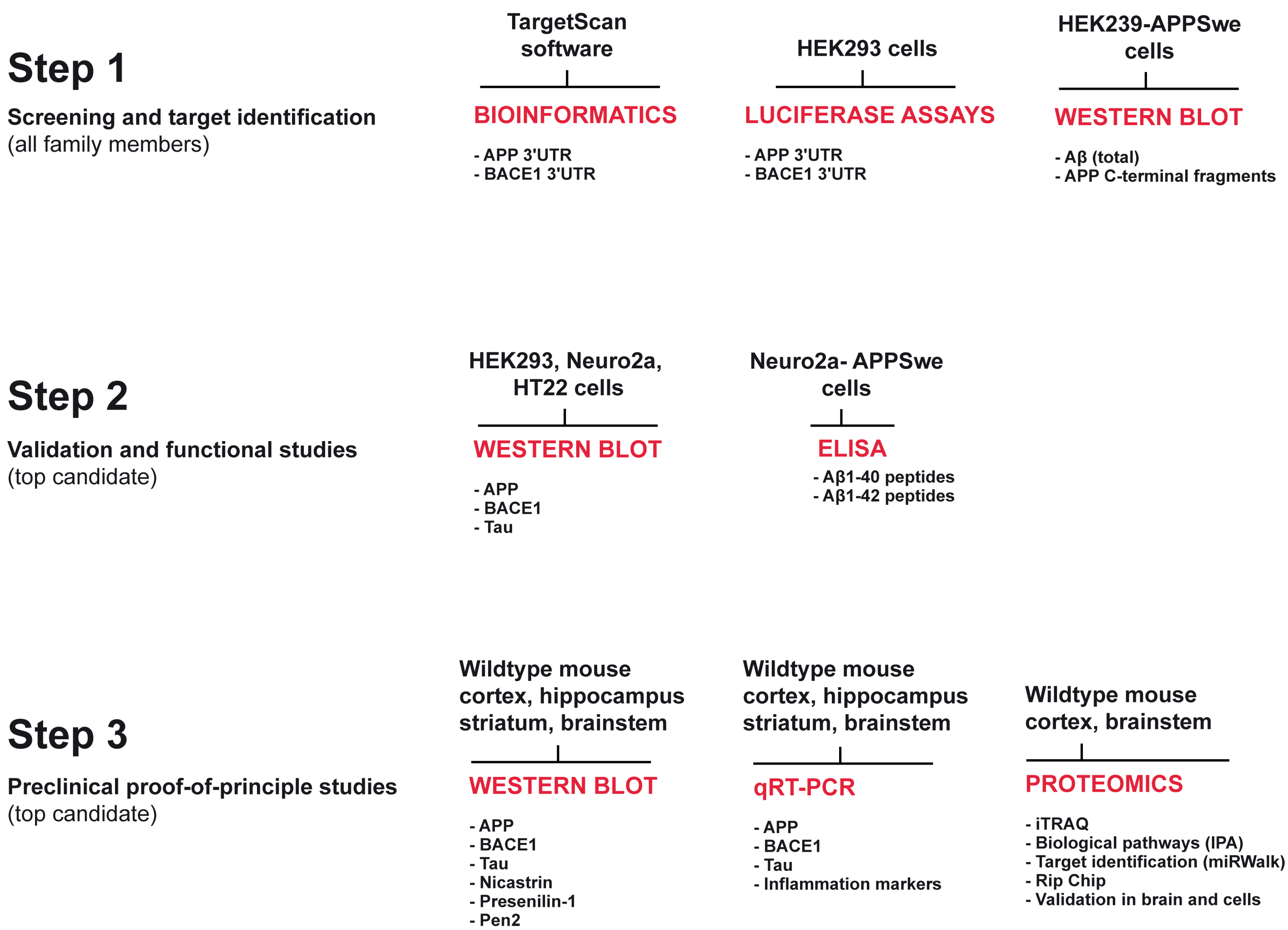
4) Supplementary Tables and legends

SUPPLEMENTARY METHODS

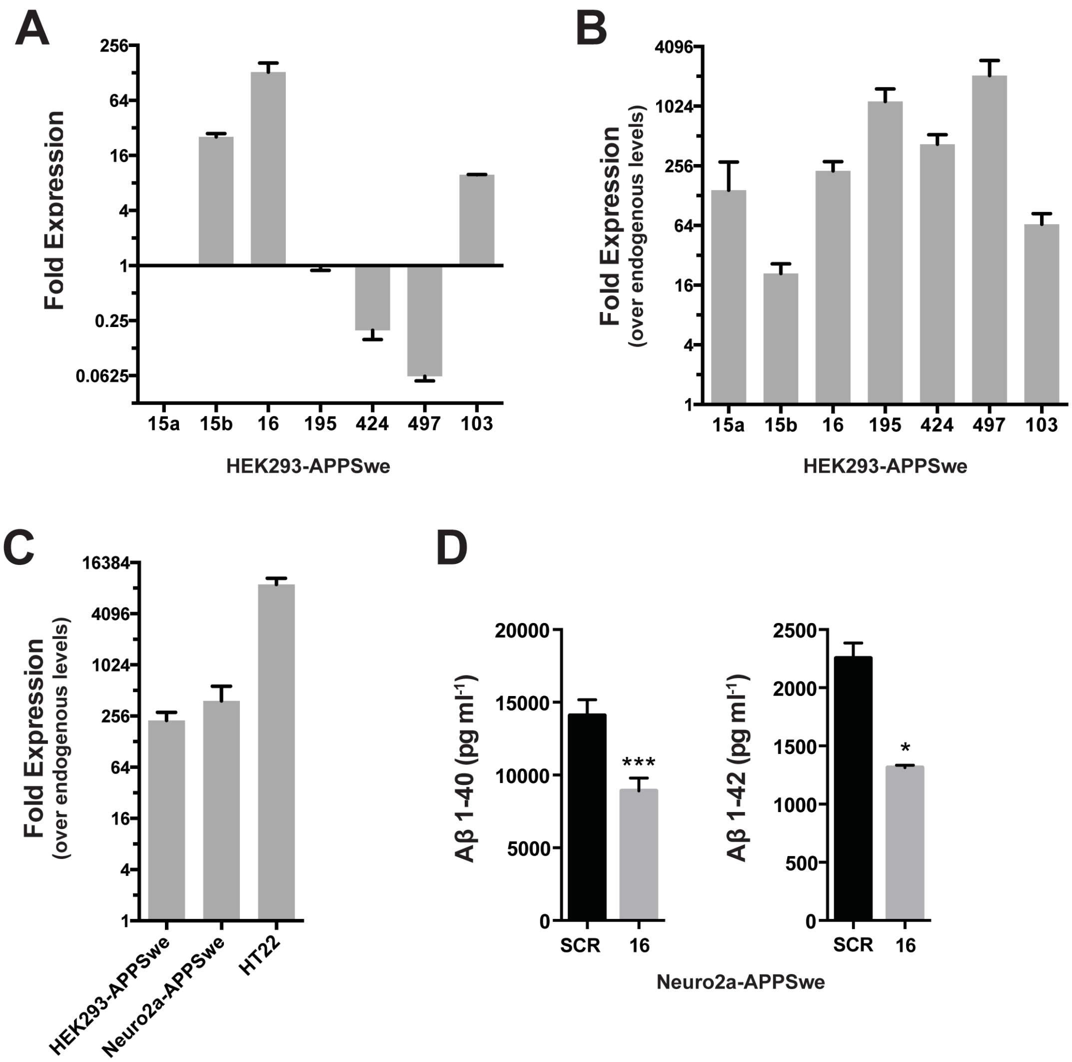
Patient information

All human and mouse studies were approved by the national ethical committee protocols and in agreement with the Université Laval ethical committee. Brain tissue from patients came from the Douglas Bell Canada Brain Bank, Montreal, Canada, and included non-dementia controls and AD cases, based on neuropathological diagnosis. Patient information is available elsewhere [1, 2]. Blocks of tissue from temporal cortex, prefrontal cortex and hippocampus were dissected and snap frozen in liquid nitrogen until use.

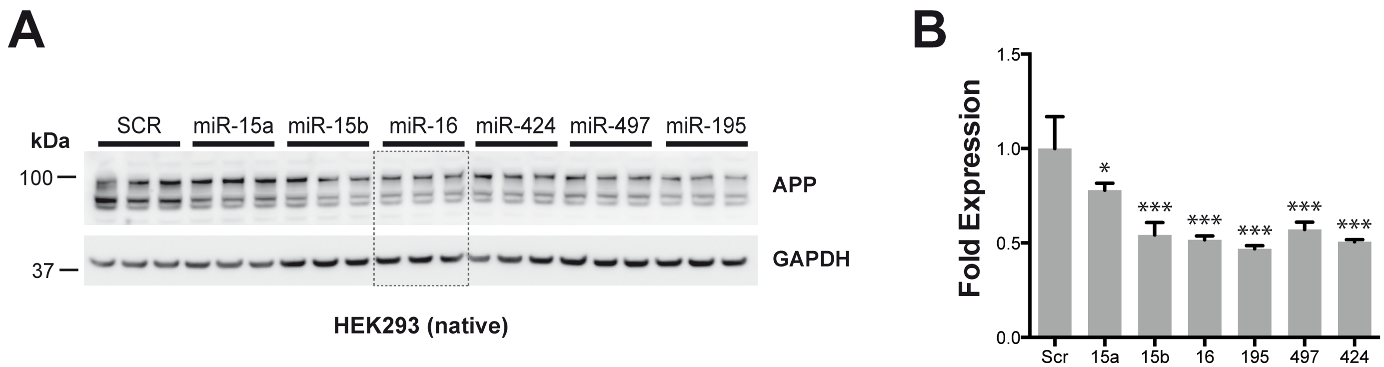
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Experimental overview of current study.

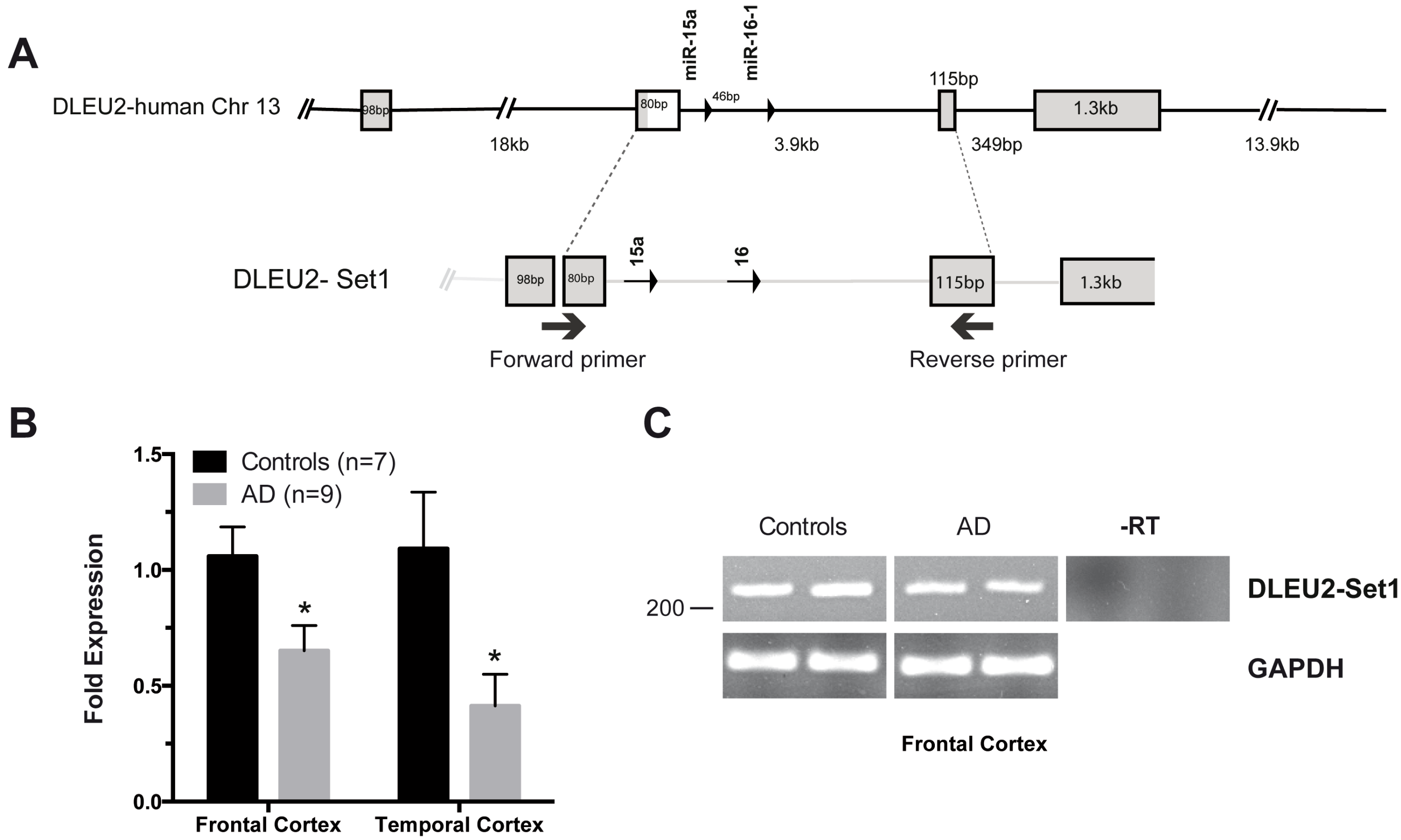


Supplementary Figure 2. **(A)** Endogenous miR-15a, miR-15b, miR-16, miR-195, miR-424, miR-497 and miR-103 levels were quantified by qRT-PCR in HEK293-APPSwe cells. U48 small nucleolar RNA (RNU48) was used as normalizing control. The relative expression was calculated using the ΔΔCt method (using miR-15a as 1 fold). **(B)** qRT-PCR analysis of ectopic miR-16 family members following transfection in HEK293-APPSwe cells. Relative expression is shown (using endogenous miRNAs as 1 fold). RNU48 was used as normalization control. **(C)** qRT-PCR analysis of transfected miR-16 in various cells lines used in this study. Relative quantifications are shown (using endogenous miR-16 as 1 fold). RNU48 was used as normalization control. **(D)** ELISA of soluble Aβ40 and Aβ42 in Neuro2a-APPSwe cells transfected with miR-16 or SCR mimics. Measurements were done 48h post-transfection. Statistical significance was determined by a *Student paired t* test (* = $p<0.05$, *** = $p<0.001$). All data are shown as mean± SEM from two or more independent experiments in triplicate.

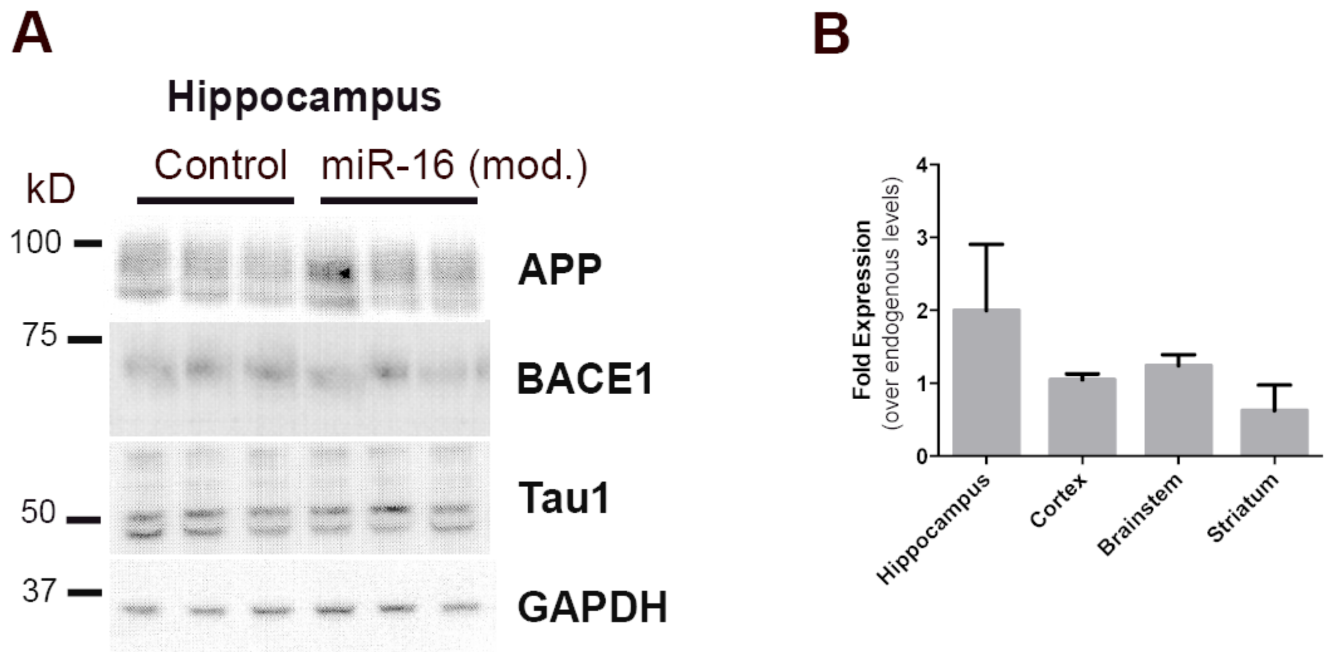


Supplementary Figure 3.

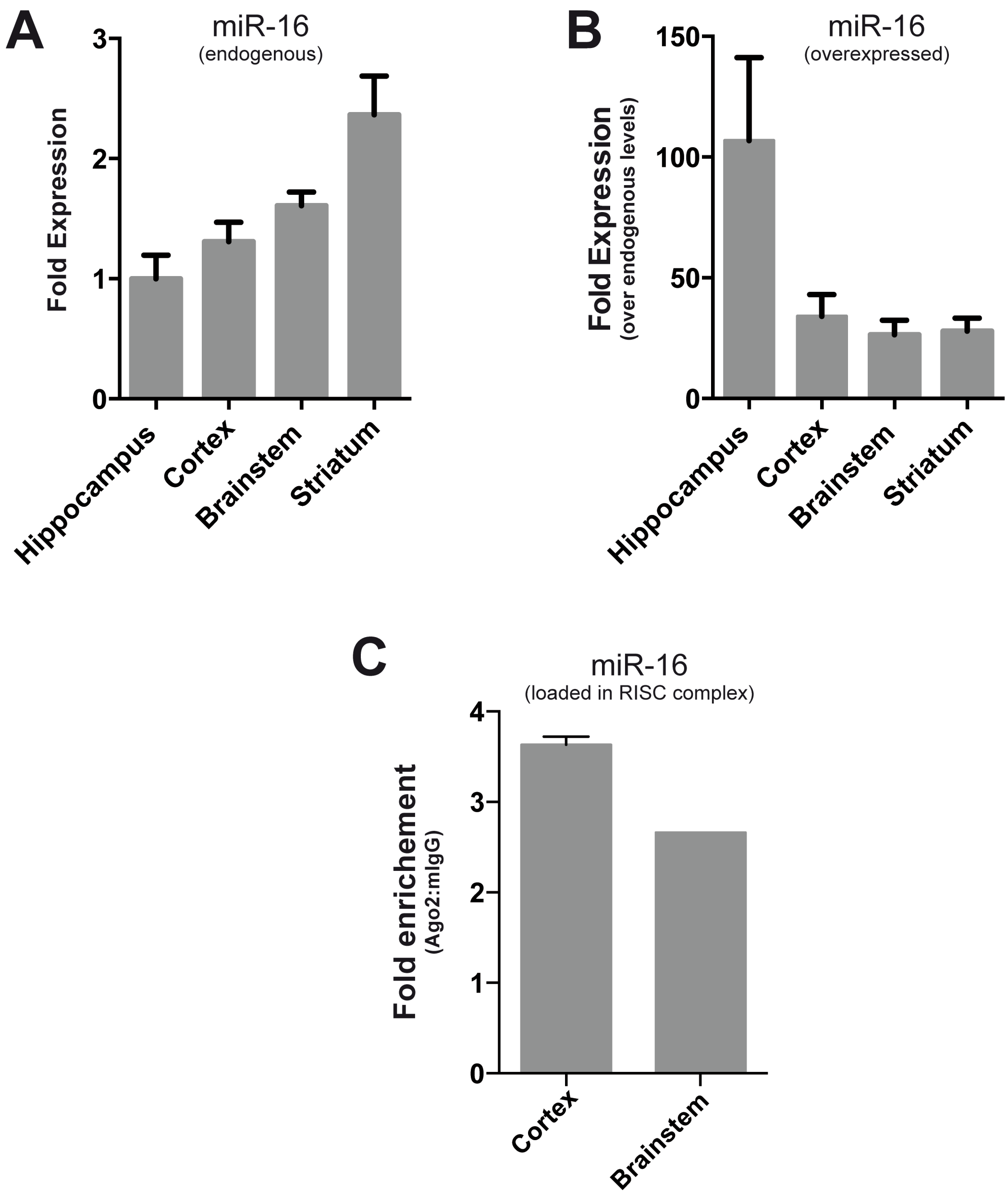
(A, B) Western blot analysis of endogenous full-length APP in native HEK293 cells following mimic overexpression at 50 nM final concentration. Shown here are results at 48h post-transfection. Statistical significance was determined by a *Student paired t* test (* = $p<0.05$, *** = $p<0.001$). Data are shown as mean± SEM from two experiments performed in triplicate. Quantifications are shown using Gapdh as normalization control.



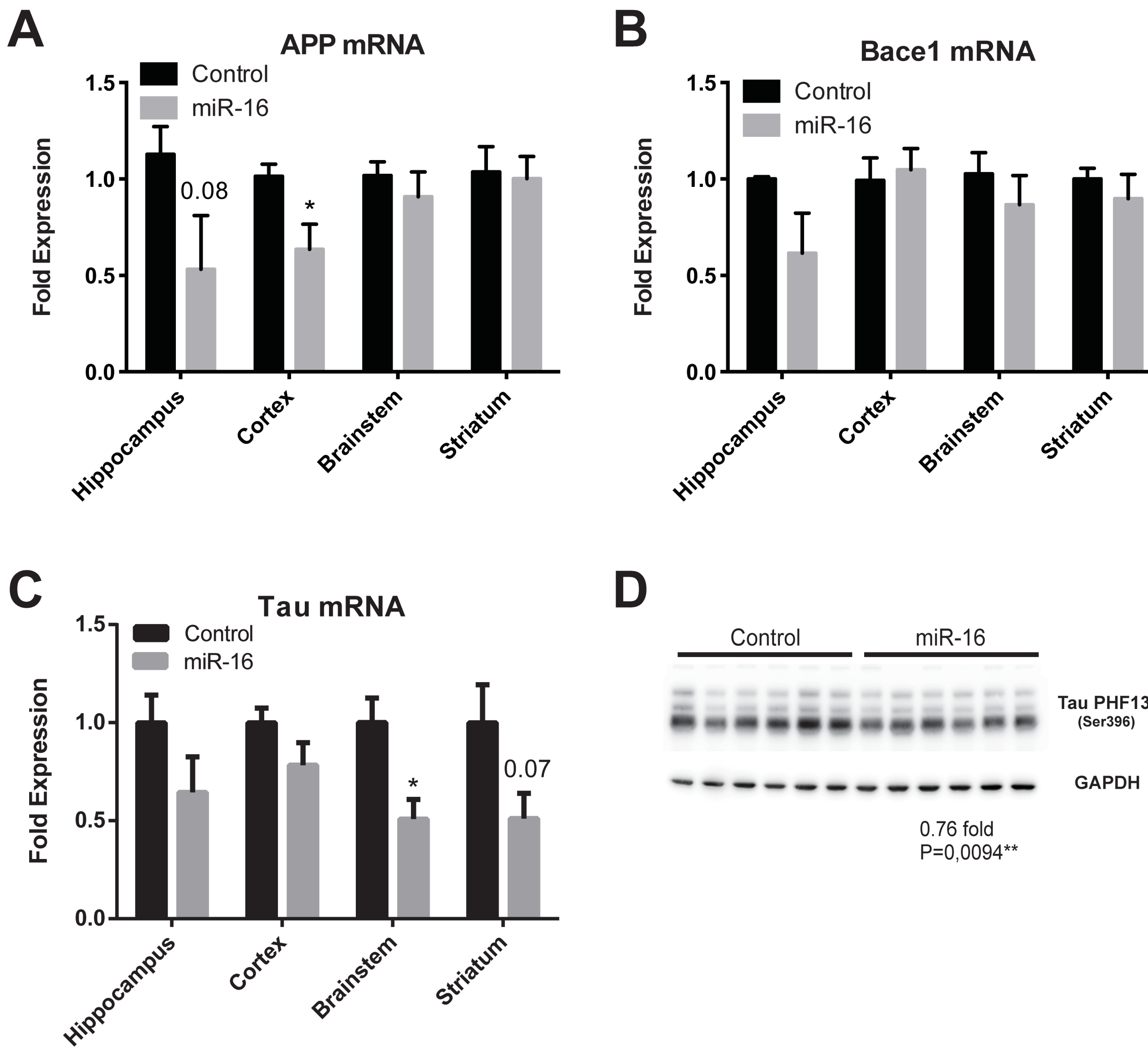
Supplementary Figure 4. Downregulation of the miR-16/15a cluster in AD. **(A)** Schematic representation (not to scale) of the DLEU2 transcript encoding miR-15a and miR-16-1 (upper panel). Close-up of the amplified region (lower panel). Primer sequences are Forward: CTCAGCAATTCTTACCTTTCTTAC; Reverse: TTCCTGGATACTCTCCTGTAGTC. **(B)** qRT-PCR of DLEU2 mRNA from non-demented Controls (N=7) and AD individuals (N=9). Relative expression is shown (using Controls as 1 fold). RNU48 was used as normalization control. All samples were measured in triplicate. Statistical significance was determined by a *Mann-Whitney U* test (* = $p<0.05$). **(C)** Validation by conventional PCR in frontal cortex tissue. Shown here are two control and two AD individuals. Minus (-) RT was used as PCR negative control.



Supplementary Figure 5. Validation of miR-16 mimic specificity *in vivo*. **(A)** Representative western blot analysis of mice treated with chemically-modified miR-16 mimics (miR-16 mod.), harbouring 2O-Me modifications on both sense and antisense stands (50µg/day for 7 days, n=8 mice/group). Shown here is the hippocampus. Similar negative results were obtained in the cortex, brainstem and striatum. Control mice received vehicle alone (0.9% saline) **(B)** qRT-PCR analysis of miR-16 mod.-treated mice. These results indicate that miR-16 expression levels are not significantly increased following treatment (n=8/group), consistent with the notion that modified mimics are not functional. Data are shown as mean± SEM.



Supplementary Figure 6. (A) Levels of endogenous miR-16 in the different brain regions of control mice (i.e., baseline levels). These experiments were performed from control (saline) treated mice. **(B)** qRT-PCR analysis showing ectopic miR-16 expression and distribution following mimic delivery. These results indicated a strong increase in miR-16 levels (over endogenous levels) in the hippocampus (106 fold), cortex (34 fold), striatum (27 fold), and brainstem (27 fold). **(C)** RIP-Chip was performed on cortex and brainstem of miR-16 mimic-treated mice (n=3/group). We observed a 3.63 and 2.66 fold enrichment in the cortex and brainstem, respectively, compared to controls. Control mouse IgGs served as normalization control. Data are shown as mean± SEM.

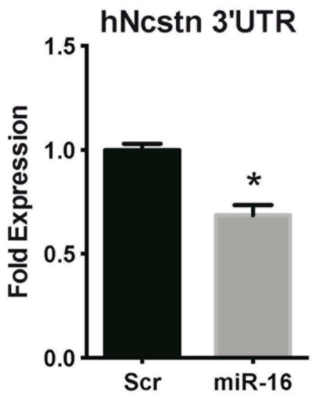


Supplementary Figure 7. Endogenous APP **(A)**, BACE1 **(B)**, and Tau **(C)** mRNA levels following miR-16 mimic treatment. mRNA levels were measured by qRT-PCR (n=6/group). Statistical significance was assessed by parametric unpaired t test with Welch's correction, P<0.05 considered as statistical significant. GAPDH served as normalization control. Data are shown as mean± SEM. **(D)** Representative western blot of endogenous Tau (PHF1 epitope) following miR-16 mimic treatment (N=8/group). Blots were normalized to Gapdh. Statistical significance was determined by a *Student paired t* test.

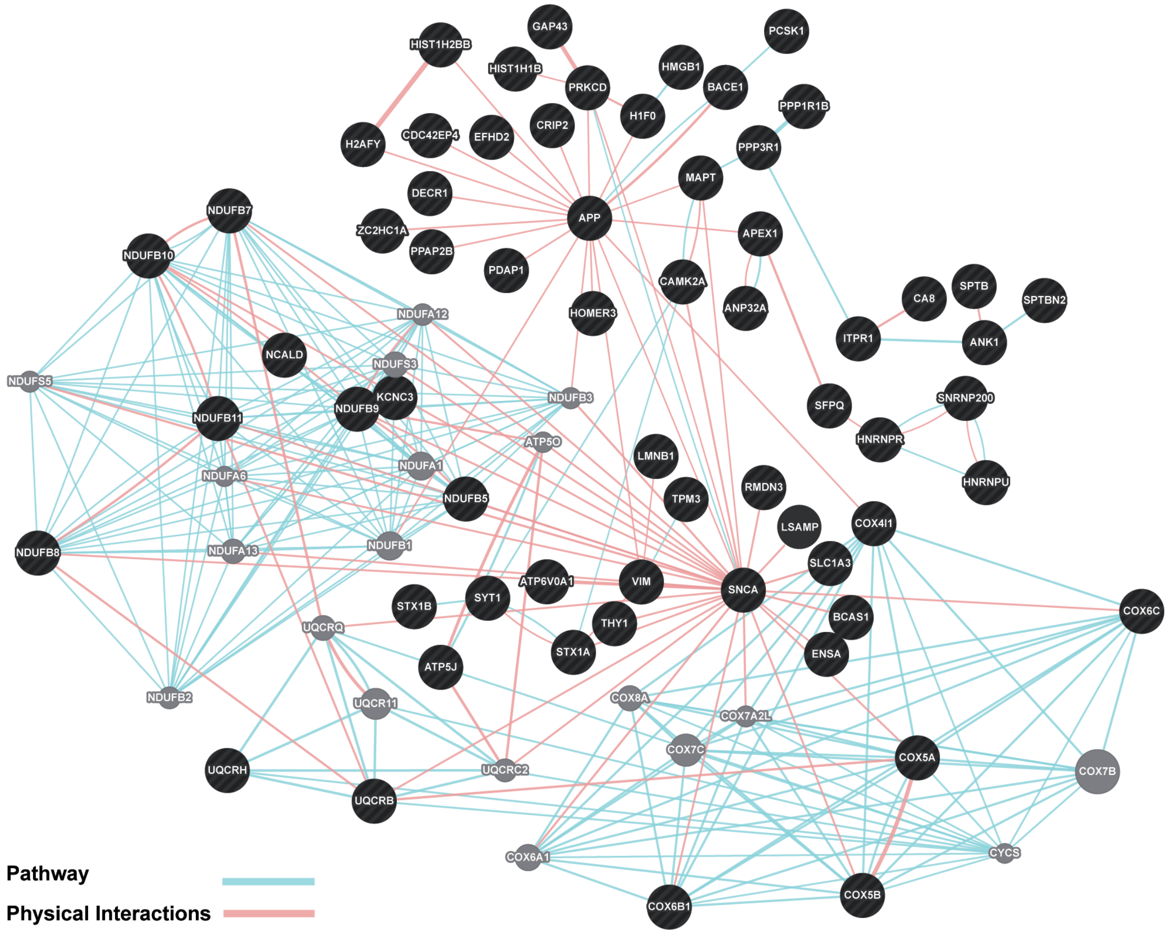
A

Gene	MicroRNA	DIANAmT	miRanda	miRDB	miRWalk	RNAhybrid	PICTAR5	PITA	RNA22	Targetscan	SUM
NCSTN	hsa-miR-16	1	0	0	0	0	0	0	1	1	3
Ncstn	mmu-miR-16	1	1	0	1	0	1	0	0	1	5

B



Supplementary Figure 8. Nicastrin is directly regulated by miR-16. **(A)** Comparative bioinformatics analysis of putative miR-16 binding sites within the mouse or human Nicastrin 3'UTR. Results were taken from the miRWalk program. **(B)** Luciferase assay on wildtype human Nicastrin 3'UTR co-transfected with 50nM mimics (SCR or miR-16) in HEK293T cells. The cells were lysed 24hrs post-transfection and luciferase signal was measured (n=2 in triplicate). Statistical significance was assessed by one-way ANOVA with Bonferroni post-test (*$p < 0.05$).



Supplementary Figure 9. Physical interaction networks between putative miR-16 targets *in vivo*. Analysis was performed using the Germania software. Both up- and down-regulated proteins were used in these analyses. Black nodes indicate proteins identified in our proteomics analysis. Grey nodes indicate additional putative binding partners in these pathways based on bioinformatics.

SUPPLEMENTARY REFERENCES

1. Smith, P, Al Hashimi, A, Girard, J, Delay, C, and Hebert, SS (2011). In vivo regulation of amyloid precursor protein neuronal splicing by microRNAs. *Journal of neurochemistry* **116**: 240-247.

2. Hebert, SS, Papadopoulou, AS, Smith, P, Galas, MC, Planel, E, Silahtaroglu, AN*, et al.* (2010). Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. *Human molecular genetics* **19**: 3959-3969.

SUPPLEMENTARY TABLES

Supplementary Table 1. Protein changes in the brainstem and hippocampus of treated mice versus controls (n=4/group). A total of 103 proteins were significantly changed in the brainstem, including 47 upregulated (in red) and 55 downregulated (in green) proteins. A total of 16 proteins were misregulated in the hippocampus, including 5 upregulated (in red) and 11 downregulated (in green) proteins (fold change <0.8 and >1.2, P <0.05). Bioinformatics predictions were done by miRWalk.

Supplementary Table 2. DAVID gene enrichment analysis of top ranked targets identified in brainstem using Homo sapiens background.

Supplementary Table 1

Brainstem								Predicted miR-16 target site	
No Accession	Gene name	Description	Score	95% Coverage	Number of peptides	RATIO mimics vs controls (brainstem)	p-value	3'UTR	Coding region
Q91XV3	**BASP1**	BASP1_MOUSE Brain acid soluble protein 1	82,29	93%	106	0,501	1,65E-07		
O35526	**STX1A**	STX1A_MOUSE Syntaxin-1A	24,07	56%	19	0,586	8,92E-06		
P19536	**COX5B**	COX5B_MOUSE Cytochrome c oxidase subunit 5B, mitochondrial	21,66	53%	16	0,603	0,00121697		
P12787	**COX5A**	COX5A_MOUSE Cytochrome c oxidase subunit 5A, mitochondrial	39,45	69%	33	0,614	0,000160624		
Q53YX2	**Thy1**	Q53YX2_MOUSE CD90.1	12,01	33%	19	0,618	0,00277828		
Q1MX42	**Prkcd**	Q1MX42_MOUSE Protein kinase C delta type	7,59	9%	5	0,65	0,0326449		
Q3TPT3	**Syt1**	Q3TPT3_MOUSE Putative uncharacterized protein	53,45	58%	48	0,653	9,97E-05		
A2AQ25-4	**Skt**	A2AQ25-4 Isoform 4 of Sickle tail protein	11,19	7%	8	0,673	0,00476251		
Q9D6J5	**Ndufb8**	NDUB8_MOUSE NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial	10	38%	9	0,674	0,00526244		
Q4VBC9	**Ndufb11**	Q4VBC9_MOUSE Ndufb11 protein (Fragment)	10,71	41%	6	0,679	0,0095815		
Q9CPQ1	**COX6C**	COX6C_MOUSE Cytochrome c oxidase subunit 6C	17,85	63%	11	0,685	0,000203329		
P56391	**Cox6b1**	CX6B1_MOUSE Cytochrome c oxidase subunit 6B1	13,62	70%	20	0,685	0,00171406		
Q9D855	**QCR7**	QCR7_MOUSE Cytochrome b-c1 complex subunit 7	17,77	57%	15	0,695	0,000153512		
P31650	**Slc6a11**	S6A11_MOUSE Sodium- and chloride-dependent GABA transporter 3	20,84	16%	12	0,698	0,00502416		
Q9JKC6	**Cend1**	CEND_MOUSE Cell cycle exit and neuronal differentiation protein 1	15,54	57%	19	0,7	0,000423472		
Q60829	**Ppp1r1b**	PPR1B_MOUSE Protein phosphatase 1 regulatory subunit 1B	18	64%	12	0,702	0,004223		
P19783	**Cox4i1**	COX41_MOUSE Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	14,86	43%	19	0,706	0,0155639		
P06837	**Gap43**	NEUM_MOUSE Neuromodulin	36,36	71%	32	0,708	5,42E-07		
Q9CQH3	**Ndufb5**	NDUB5_MOUSE NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial	10	21%	6	0,711	0,0077551		
Q6ZWX2	**Tmsb4x**	Q6ZWX2_MOUSE Thymosin, beta 4, X chromosome	12,85	77%	11	0,712	0,0218587		
Q9CR61	**Ndufb7**	NDUB7_MOUSE NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7	14,31	58%	8	0,718	0,0129444		
Q8BGZ1	**Hpcal4**	HPCL4_MOUSE Hippocalcin-like protein 4	18,91	81%	23	0,722	0,000985372		
Q9DCT8	**CRIP2**	CRIP2_MOUSE Cysteine-rich protein 2	10,28	39%	7	0,723	0,0114881		
Q8BLF7	**Slc2a3**	Q8BLF7_MOUSE Putative uncharacterized protein	16,87	13%	11	0,731	0,00105785		
P11798	**Camk2a**	KCC2A_MOUSE Calcium/calmodulin-dependent protein kinase type II subunit alpha	59,38	50%	75	0,731	0,00178941		
Q9QXV0	**PCSK1**	PCSK1_MOUSE ProSAAS	16,38	47%	12	0,732	0,000728901		
Q91X97	**NCALD**	NCALD_MOUSE Neurocalcin-delta	14,01	74%	25	0,735	0,0215997		
Q99JY8	**LPP3**	LPP3_MOUSE Lipid phosphate phosphohydrolase 3	11,31	17%	8	0,74	0,0151303		
Q80YN3	**BCAS1**	BCAS1_MOUSE Breast carcinoma-amplified sequence 1 homolog	30,42	27%	23	0,743	1,08E-05		
Q3TYE5	**Lsamp**	Q3TYE5_MOUSE Limbic system-associated membrane protein	20,73	36%	13	0,749	0,0426762		
P60840	**ENSA**	ENSA_MOUSE Alpha-endosulfine	13,51	70%	12	0,749	0,00174027		
P99028	**QCR6**	QCR6_MOUSE Cytochrome b-c1 complex subunit 6, mitochondrial	12,35	60%	8	0,756	0,0278268		
Q3UHX2	**Pdap1**	HAP28_MOUSE 28 kDa heat- and acid-stable phosphoprotein	12,59	34%	9	0,757	0,00129728		
P51830	**ADCY9**	ADCY9_MOUSE Adenylate cyclase type 9	6,95	4%	5	0,759	0,0321568		
O54983	**CRYM**	CRYM_MOUSE Thiomorpholine-carboxylate dehydrogenase	27,14	51%	23	0,761	0,0168747		
P97450	**ATP5J**	ATP5J_MOUSE ATP synthase-coupling factor 6, mitochondrial	13,72	56%	12	0,763	0,00373859		
P63321	**RALA**	RALA_MOUSE Ras-related protein Ral-A	16	43%	11	0,765	0,0349347		
Q8BLE7	**Slc17a6**	VGLU2_MOUSE Vesicular glutamate transporter 2	12,05	17%	8	0,765	0,00936656		
Q9CQZ1	**HSBP1**	HSBP1_MOUSE Heat shock factor-binding protein 1	8	80%	6	0,765	0,0386879		
O55042	**Snca**	SYUA_MOUSE Alpha-synuclein	26,02	91%	31	0,766	0,0013887		
Q63810-2	**Ppp3r1**	Q63810-2 Isoform 2 of Calcineurin subunit B type 1	26,06	77%	28	0,769	0,00459909		
Q9D8B3	**Chmp4b**	CHM4B_MOUSE Charged multivesicular body protein 4b	10,14	29%	9	0,77	0,0217823		
Q03517	**SCG2**	SCG2_MOUSE Secretogranin-2	46,68	48%	26	0,775	2,77E-07		
Q9DCS9	**Ndufb10**	NDUBA_MOUSE NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	18,01	57%	11	0,777	0,0128557		
Q9Z1G4-3	**Atp6v0a1**	Q9Z1G4-3 Isoform A1-III of V-type proton ATPase 116 kDa subunit a isoform 1	47,89	30%	42	0,783	5,00E-07		
B1AS06	**Dlgap3**	B1AS06_MOUSE Disks large-associated protein 3	5,9	5%	5	0,785	0,0338111		
Q8C845	**Efhd2**	Q8C845_MOUSE EF-hand domain-containing protein D2	24,4	51%	18	0,788	0,000307613		
P31324	**KAP3**	KAP3_MOUSE cAMP-dependent protein kinase type II-beta regulatory subunit	35,3	52%	18	0,788	0,00215375		
Q8BJH1	**Zc2hc1a**	ZC21A_MOUSE Zinc finger C2HC domain-containing protein 1A	12,01	19%	7	0,79	0,0404972		
Q9ER00	**STX12**	STX12_MOUSE Syntaxin-12	15,15	35%	11	0,79	0,0119414		
P61264	**STX1B**	STX1B_MOUSE Syntaxin-1B	43,3	56%	38	0,79	1,78E-05		
Q9CQJ8	**Ndufb9**	NDUB9_MOUSE NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9	16,19	48%	10	0,792	0,0217948		
Q3U2J2	**Slc2a1**	Q3U2J2_MOUSE Putative uncharacterized protein	10,01	11%	5	0,793	0,0463827		
Q58E70	**Tpm3**	Q58E70_MOUSE Tpm3 protein	18	49%	22	0,795	0,0242466		
A2A7R6	**Hpca**	A2A7R6_MOUSE Neuron-specific calcium-binding protein hippocalcin (Fragment)	36,76	79%	31	0,795	0,0258137		
Q8BTS0	**Ddx5**	Q8BTS0_MOUSE DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	45,74	44%	29	1,199	0,0023451		
Q9Z1N5	**Ddx39b**	DX39B_MOUSE Spliceosome RNA helicase Ddx39b	27,42	35%	17	1,2	0,00233304		
Q8VIJ6	**Sfpq**	SFPQ_MOUSE Splicing factor, proline- and glutamine-rich	49,54	42%	35	1,202	0,000109032		
Q8VDN2	**Atp1a1**	AT1A1_MOUSE Sodium/potassium-transporting ATPase subunit alpha-1	66,56	52%	159	1,203	9,79E-07		
Q9CT37	**Hnrnpr**	Q9CT37_MOUSE Putative uncharacterized protein (Fragment)	12,01	31%	10	1,211	0,0458658		
Q00PI9	**Hnrnpul2**	HNRL2_MOUSE Heterogeneous nuclear ribonucleoprotein U-like protein 2	27,12	26%	17	1,213	0,00892075		
Q02357-5	**Ank1**	Q02357-5 Isoform 5 of Ankyrin-1	35,26	19%	29	1,223	2,29E-05		
Q9QZQ8-2	**H2afy**	Q9QZQ8-2 Isoform 1 of Core histone macro-H2A.1	26,07	38%	15	1,226	0,00200346		
Q3UTJ2	**Sorbs2**	SRBS2_MOUSE Sorbin and SH3 domain-containing protein 2	15,78	8%	10	1,231	0,0271742		
Q497Z6	**Hmgb1**	Q497Z6_MOUSE High mobility group box 1	15,14	37%	14	1,233	0,0157343		
Q8K0S0	**Phyhip**	PHYIP_MOUSE Phytanoyl-CoA hydroxylase-interacting protein	19,24	42%	15	1,233	0,00591662		
Q8BTM8	**Flna**	FLNA_MOUSE Filamin-A	31,45	12%	23	1,24	0,0234162		
Q8R384	**Myh11**	Q8R384_MOUSE Myh11 protein	32,51	21%	38	1,24	0,00372456		
Q8BH44	**Coro2b**	COR2B_MOUSE Coronin-2B	23,59	31%	13	1,241	0,00286535		
Q6P4T2	**Snrnp200**	Q6P4T2_MOUSE Activating signal cointegrator 1 complex subunit 3-like 1	16,42	7%	13	1,242	0,0415583		
O35381	**Anp32a**	AN32A_MOUSE Acidic leucine-rich nuclear phosphoprotein 32 family member A	20,06	31%	15	1,242	0,0113832		
Q9JMF3	**Gng13**	GBG13_MOUSE Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-13	6,61	46%	5	1,252	0,0364961		
Q9CQ62	**Decr1**	DECR_MOUSE 2,4-dienoyl-CoA reductase, mitochondrial	14,11	24%	7	1,255	0,0164508		
Q3UJU9	**Rmdn3**	RMD3_MOUSE Regulator of microtubule dynamics protein 3	13,61	22%	9	1,258	0,0129001		
Q3UGY4	**Sptb**	Q3UGY4_MOUSE Putative uncharacterized protein	71,35	29%	57	1,262	6,69E-06		
A2A5R2	**Arfgef2**	BIG2_MOUSE Brefeldin A-inhibited guanine nucleotide-exchange protein 2	8,74	4%	6	1,264	0,0346823		
G3XA10	**Hnrnpu**	G3XA10_MOUSE Heterogeneous nuclear ribonucleoprotein U	36,21	31%	25	1,264	5,20E-05		
D3YZV4	**Kcnc3**	D3YZV4_MOUSE Potassium voltage-gated channel subfamily C member 3	11,6	13%	6	1,28	0,0275812		
P14733	**Lmnb1**	LMNB1_MOUSE Lamin-B1	46,05	44%	28	1,285	2,81E-06		
Q68FG2	**sptbn2**	Q68FG2_MOUSE Protein Sptbn2	171,89	58%	139	1,286	1,17E-16		
E0CXN5	**Gpd1**	E0CXN5_MOUSE Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	44,7	68%	25	1,29	0,00073079		
Q3UHH0	**Atp2b2**	Q3UHH0_MOUSE Putative uncharacterized protein	80,6	39%	64	1,292	3,26E-07		
Q80VD1	**Fam98b**	FA98B_MOUSE Protein FAM98B	9,85	17%	5	1,297	0,0259779		
Q3U4Y0	**H1f0**	Q3U4Y0_MOUSE Putative uncharacterized protein	12	27%	9	1,297	0,041291		
P20152	**Vim**	VIME_MOUSE Vimentin	68,44	75%	60	1,316	5,50E-06		
Q9WVK4	**Ehd1**	EHD1_MOUSE EH domain-containing protein 1	33,52	62%	29	1,333	0,000632995		
Q8C413	**Dgkg**	Q8C413_MOUSE Diacylglycerol kinase gamma	18,4	19%	10	1,347	0,00102144		
P28352	**Apex1**	APEX1_MOUSE DNA-(apurinic or apyrimidinic site) lyase	16,05	38%	10	1,466	0,0323024		
UPI00000E8E77		unknown [Mus musculus [10090]]	12,65	25%	8	1,512	0,000560151		
P32848	**Pvalb**	PRVA_MOUSE Parvalbumin alpha	20	77%	12	1,518	0,000198945		
Q99NG1	**Slc1a3**	Q99NG1_MOUSE Glutamate/aspartate transporter (Fragment)	21,1	22%	36	1,532	0,0213031		
Q9JM96	**Cdc42ep4**	BORG4_MOUSE Cdc42 effector protein 4	10	16%	5	1,549	0,0279819		
P43276	**Hist1h1b**	H15_MOUSE Histone H1.5	8,21	19%	5	1,714	0,02542		
Q64475	**Hist1h2bb**	H2B1B_MOUSE Histone H2B type 1-B	16,08	43%	34	1,722	0,0243785		
Q6ZQK5-2	**Acap2**	Q6ZQK5-2 Isoform 2 of Arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 2	6,39	7%	6	1,731	0,0222356		
Q8BQ28	**Nck2**	Q8BQ28_MOUSE Putative uncharacterized protein	4,96	11%	3	1,735	0,0211423		
P37804	**Tagln**	TAGL_MOUSE Transgelin	18,77	52%	11	1,751	0,000255131		
P12658	**Calb1**	CALB1_MOUSE Calbindin	25,76	67%	24	2,202	4,99E-08		
P11881-8	**Itpr1**	P11881-8 Isoform 8 of Inositol 1,4,5-trisphosphate receptor type 1	71,39	19%	48	3,208	6,43E-19		
Q99JP6-2	**Homer3**	Q99JP6-2 Isoform 2 of Homer protein homolog 3	9,84	22%	6	3,396	0,000109871		
Q3TXM3	**Slc1a6**	Q3TXM3_MOUSE Putative uncharacterized protein	8	17%	10	3,784	0,000775311		
P28651	**Ca8**	CAH8_MOUSE Carbonic anhydrase-related protein	12,02	33%	6	4,709	6,54E-05		

Hippocampus								Predicted miR-16 target site	
No Accession	gene name	Description	Score	95% Converage	Number of peptides	RATIO mimics vs. control (hippocampus)	p-value	3'UTR	Coding region
P31725	**S100a9**	S10A9_MOUSE Protein S100-A9	6	30%	4	0,471	0,0039529		
Q8C872	**Tfrc**	Q8C872_MOUSE Transferrin receptor protein 1	4,39	7%	3	0,666	0,0373557		
Q8K0E8	**Fgb**	FIBB_MOUSE Fibrinogen beta chain	15	19%	8	0,671	0,0414647		
Q8C8M3	**Stat1**	Q8C8M3_MOUSE Putative uncharacterized protein	10,44	13%	7	0,714	0,00461793		
Q99K47	**Fga**	Q99K47_MOUSE Fibrinogen, alpha polypeptide	10,34	11%	5	0,736	0,00496785		
F6X7Z3	**Cpeb1**	F6X7Z3_MOUSE Cytoplasmic polyadenylation element-binding protein 1 (Fragment)	5,69	13%	3	0,747	0,0253883		
Q9QYT9	**Prnpb**	Q9QYT9_MOUSE Major prion protein	8,41	22%	7	0,768	0,030742		
Q6PAR5-6	**Gapvd1**	Q6PAR5-6 Isoform 6 of GTPase-activating protein and VPS9 domain-containing protein 1	8,01	3%	5	0,771	0,0382554		
Q91X72	**Hpx**	HEMO_MOUSE Hemopexin	14,38	21%	7	0,78	0,00303796		
Q3UVL4	**Vps51**	VPS51_MOUSE Vacuolar protein sorting-associated protein 51 homolog	13,57	13%	8	0,785	0,0111839		
Q8BTI8	**Srrm2**	SRRM2_MOUSE Serine/arginine repetitive matrix protein 2	3,29	1%	4	0,798	0,0231391		
Q80TQ3	**Nefh**	Q80TQ3_MOUSE MKIAA0845 protein (Fragment)	68,1	34%	61	1,306	0,00336826		
P07309	**Ttr**	TTHY_MOUSE Transthyretin	8	30%	5	1,411	0,0244789		
P28651	**Ca8**	CAH8_MOUSE Carbonic anhydrase-related protein	12,02	33%	6	1,469	0,0400057		
Q925J6	**Ptprn**	Q925J6_MOUSE Protein tyrosine phosphatase receptor type N (Fragment)	5,24	4%	3	1,525	0,0386948		
Q3TCQ7	**Tsc2**	Q3TCQ7_MOUSE Putative uncharacterized protein	3,49	2%	4	1,799	0,0208798		

Uniref http://www.uniprot.org/help/uniref mus musculus

Supplementary Table 2

Background: Homo sapiens

Category	Term	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Alzheimer's disease	14	15.1	5.1E-10	3.0E-8
	Parkinson's disease	12	12.9	6.3E-9	1.9E-7
	Oxidative phosphorylation	12	12.9	7.5E-9	1.5E-7
	Huntington's disease	12	12.9	2.2E-7	3.3E-6
	Cardiac muscle contraction	7	7.5	4.8E-5	5.6E-4
	SNARE interactions in vesicular transport	3	3.2	4.2E-2	3.5E-1
	GnRH signaling pathway	4	4.3	5.2E-2	3.6E-1
	Calcium signaling pathway	5	5.4	6.4E-2	3.9E-1
	Oocyte meiosis	4	4.3	6.9E-2	3.7E-1
	Vascular smooth muscle contraction	4	4.3	7.2E-2	3.6E-1

Category	Term	Count	%	P-Value	Benjamini
GOTERM_BP_FAT	oxidative phosphorylation	8	8.6	1.7E-6	1.7E-3
	respiratory electron transport chain	7	7.5	2.0E-6	9.8E-4
	generation of precursor metabolites and energy	12	12.9	2.2E-6	7.0E-4
	electron transport chain	8	8.6	4.8E-6	1.2E-3
	mitochondrial ATP synthesis coupled electron transport	6	6.5	1.9E-5	3.7E-3
	ATP synthesis coupled electron transport	6	6.5	1.9E-5	3.7E-3
	cellular respiration	7	7.5	2.3E-5	3.7E-3
	mitochondrial electron transport, NADH to ubiquinone	5	5.4	1.1E-4	1.5E-2
	neurotransmitter transport	6	6.5	1.3E-4	1.5E-2
	regulation of cellular component biogenesis	7	7.5	1.9E-4	2.0E-2
	energy derivation by oxidation of organic compounds	7	7.5	2.1E-4	2.0E-2
	regulation of synaptic transmission	6	6.5	1.3E-3	1.0E-1
	oxidation reduction	12	12.9	1.3E-3	9.9E-2
	negative regulation of cellular component organization	6	6.5	1.5E-3	1.1E-1
	regulation of transmission of nerve impulse	6	6.5	1.8E-3	1.2E-1
	regulation of protein complex assembly	5	5.4	2.0E-3	1.2E-1
	regulation of neurological system process	6	6.5	2.1E-3	1.2E-1
	regulation of system process	8	8.6	2.3E-3	1.2E-1
	cellular macromolecular complex assembly	8	8.6	2.7E-3	1.4E-1
	regulation of cellular localization	7	7.5	3.5E-3	1.6E-1
	regulation of actin filament polymerization	4	4.3	4.0E-3	1.8E-1
	cellular macromolecular complex subunit organization	8	8.6	5.1E-3	2.1E-1
	regulation of actin polymerization or depolymerization	4	4.3	5.6E-3	2.2E-1
	regulation of actin filament length	4	4.3	6.1E-3	2.3E-1
	regulation of synaptic plasticity	4	4.3	6.4E-3	2.3E-1
	regulation of neurotransmitter secretion	3	3.2	6.7E-3	2.3E-1
	vesicle-mediated transport	10	10.8	6.8E-3	2.2E-1
	regulation of secretion	6	6.5	6.9E-3	2.2E-1
	regulation of neurotransmitter levels	4	4.3	7.3E-3	2.2E-1
	regulation of protein polymerization	4	4.3	7.6E-3	2.2E-1
	synaptic transmission	7	7.5	8.4E-3	2.4E-1
	regulation of neurotransmitter transport	3	3.2	1.0E-2	2.7E-1
	positive regulation of transport	6	6.5	1.0E-2	2.7E-1
	actin cytoskeleton organization	6	6.5	1.1E-2	2.7E-1
	purine nucleotide biosynthetic process	5	5.4	1.1E-2	2.8E-1
	negative regulation of actin filament polymerization	3	3.2	1.2E-2	2.8E-1
	protein stabilization	3	3.2	1.2E-2	2.8E-1
	nitrogen compound biosynthetic process	7	7.5	1.2E-2	2.9E-1
	negative regulation of organelle organization	4	4.3	1.3E-2	2.8E-1
	negative regulation of protein polymerization	3	3.2	1.3E-2	2.8E-1
	nucleosome assembly	4	4.3	1.3E-2	2.9E-1
	actin filament-based process	6	6.5	1.4E-2	2.9E-1
	cytoskeleton organization	8	8.6	1.5E-2	2.9E-1
	chromatin assembly	4	4.3	1.5E-2	2.9E-1
	ATP biosynthetic process	4	4.3	1.6E-2	3.0E-1
	regulation of actin cytoskeleton organization	4	4.3	1.6E-2	3.0E-1
	macromolecular complex assembly	10	10.8	1.6E-2	3.0E-1
	protein-DNA complex assembly	4	4.3	1.7E-2	3.0E-1
	regulation of exocytosis	3	3.2	1.7E-2	3.0E-1
	negative regulation of protein complex assembly	3	3.2	1.7E-2	3.0E-1
	regulation of actin filament-based process	4	4.3	1.7E-2	3.0E-1
	transmission of nerve impulse	7	7.5	1.7E-2	3.0E-1
	nucleosome organization	4	4.3	1.8E-2	3.0E-1
	regulation of vesicle-mediated transport	4	4.3	1.9E-2	3.1E-1
	sensory perception of sound	4	4.3	2.0E-2	3.1E-1
	purine ribonucleoside triphosphate biosynthetic process	4	4.3	2.0E-2	3.2E-1
	purine nucleoside triphosphate biosynthetic process	4	4.3	2.1E-2	3.2E-1
	ribonucleoside triphosphate biosynthetic process	4	4.3	2.1E-2	3.2E-1
	regulation of cellular component size	6	6.5	2.2E-2	3.3E-1
	nucleoside triphosphate biosynthetic process	4	4.3	2.2E-2	3.3E-1
	sensory perception of mechanical stimulus	4	4.3	2.3E-2	3.3E-1
	positive regulation of neurotransmitter secretion	2	2.2	2.3E-2	3.3E-1
	macromolecular complex subunit organization	10	10.8	2.4E-2	3.3E-1
	ATP metabolic process	4	4.3	2.4E-2	3.3E-1
	purine nucleotide metabolic process	5	5.4	2.4E-2	3.3E-1
	nucleotide biosynthetic process	5	5.4	2.4E-2	3.3E-1
	cell-cell signaling	9	9.7	2.5E-2	3.3E-1
	regulation of protein stability	3	3.2	2.6E-2	3.3E-1
	nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic	5	5.4	2.7E-2	3.5E-1
	nucleobase, nucleoside and nucleotide biosynthetic process	5	5.4	2.7E-2	3.5E-1
	purine ribonucleotide biosynthetic process	4	4.3	3.2E-2	3.9E-1
	DNA packaging	4	4.3	3.2E-2	3.9E-1
	purine ribonucleoside triphosphate metabolic process	4	4.3	3.2E-2	3.9E-1
	ribonucleoside triphosphate metabolic process	4	4.3	3.3E-2	3.9E-1
	secretion by cell	5	5.4	3.4E-2	4.0E-1
	positive regulation of neurotransmitter transport	2	2.2	3.5E-2	4.0E-1
	purine nucleoside triphosphate metabolic process	4	4.3	3.6E-2	4.0E-1
	posttranscriptional regulation of gene expression	5	5.4	3.6E-2	4.0E-1
	ion transport	10	10.8	3.7E-2	4.1E-1
	ribonucleotide biosynthetic process	4	4.3	3.7E-2	4.0E-1
	chromatin assembly or disassembly	4	4.3	3.9E-2	4.2E-1
	regulation of organelle organization	5	5.4	4.0E-2	4.1E-1
	aspartate transport	2	2.2	4.1E-2	4.2E-1
	negative regulation of cytoskeleton organization	3	3.2	4.2E-2	4.2E-1
	nucleoside triphosphate metabolic process	4	4.3	4.3E-2	4.2E-1
	cation transport	8	8.6	4.5E-2	4.4E-1
	phosphorylation	10	10.8	4.6E-2	4.4E-1
	regulation of cytoskeleton organization	4	4.3	4.7E-2	4.4E-1
	purine ribonucleotide metabolic process	4	4.3	4.8E-2	4.5E-1
	ribonucleotide metabolic process	4	4.3	5.6E-2	5.0E-1
	neurotransmitter uptake	2	2.2	5.8E-2	5.0E-1
	behavior	7	7.5	5.9E-2	5.1E-1
	phosphorus metabolic process	11	11.8	6.0E-2	5.1E-1
	phosphate metabolic process	11	11.8	6.0E-2	5.1E-1
	regulation of binding	4	4.3	6.2E-2	5.2E-1
	locomotory behavior	5	5.4	7.9E-2	6.0E-1
	L-glutamate transport	2	2.2	8.0E-2	6.0E-1
	dicarboxylic acid transport	2	2.2	8.5E-2	6.2E-1
	RNA splicing	5	5.4	8.7E-2	6.3E-1
	acidic amino acid transport	2	2.2	9.1E-2	6.4E-1
	actin cytoskeleton reorganization	2	2.2	9.1E-2	6.4E-1
	signal complex assembly	2	2.2	9.1E-2	6.4E-1
	positive regulation of cellular component organization	4	4.3	9.2E-2	6.4E-1
	intracellular transport	8	8.6	9.3E-2	6.4E-1
	response to interleukin-1	2	2.2	9.6E-2	6.5E-1
	regulation of cell projection organization	3	3.2	9.7E-2	6.5E-1
	neurological system process	12	12.9	9.9E-2	6.5E-1

Category	Term	Count	%	P-Value	Benjamini
GOTERM_MF_FAT	inorganic cation transmembrane transporter activity	11	11.8	2.8E-8	8.1E-6
	cytoskeletal protein binding	16	17.2	3.6E-7	5.3E-5
	monovalent inorganic cation transmembrane transporter activity	8	8.6	3.4E-6	3.3E-4
	hydrogen ion transmembrane transporter activity	7	7.5	1.9E-5	1.4E-3
	heme-copper terminal oxidase activity	5	5.4	2.4E-5	1.4E-3
	oxidoreductase activity, acting on heme group of donors, oxygen	5	5.4	2.4E-5	1.4E-3
	oxidoreductase activity, acting on heme group of donors	5	5.4	2.4E-5	1.4E-3
	cytochrome-c oxidase activity	5	5.4	2.4E-5	1.4E-3
	NADH dehydrogenase (quinone) activity	5	5.4	1.4E-4	6.7E-3
	NADH dehydrogenase activity	5	5.4	1.4E-4	6.7E-3
	NADH dehydrogenase (ubiquinone) activity	5	5.4	1.4E-4	6.7E-3
	actin binding	10	10.8	1.7E-4	7.2E-3
	oxidoreductase activity, acting on NADH or NADPH, quinone or s	5	5.4	2.3E-4	8.4E-3
	structural constituent of cytoskeleton	5	5.4	1.1E-3	3.6E-2
	calcium ion binding	15	16.1	1.3E-3	3.9E-2
	symporter activity	6	6.5	1.6E-3	4.1E-2
	L-amino acid transmembrane transporter activity	4	4.3	1.6E-3	3.9E-2
	oxidoreductase activity, acting on NADH or NADPH	5	5.4	1.7E-3	3.8E-2
	calmodulin binding	6	6.5	1.7E-3	3.5E-2
	L-glutamate transmembrane transporter activity	3	3.2	2.0E-3	3.8E-2
	acidic amino acid transmembrane transporter activity	3	3.2	2.4E-3	4.3E-2
	solute:cation symporter activity	5	5.4	2.8E-3	4.7E-2
	cytoskeletal adaptor activity	3	3.2	2.8E-3	4.5E-2
	amino acid transmembrane transporter activity	4	4.3	5.7E-3	8.5E-2
	SNAP receptor activity	3	3.2	7.3E-3	1.0E-1
	organic acid:sodium symporter activity	3	3.2	1.0E-2	1.3E-1
	amine transmembrane transporter activity	4	4.3	1.1E-2	1.3E-1
	di-, tri-valent inorganic cation transmembrane transporter activit	3	3.2	2.4E-2	2.7E-1
	P-P-bond-hydrolysis-driven transmembrane transporter activity	4	4.3	3.9E-2	3.9E-1
	primary active transmembrane transporter activity	4	4.3	3.9E-2	3.9E-1
	solute:sodium symporter activity	3	3.2	4.1E-2	3.9E-1
	enzyme binding	8	8.6	4.2E-2	3.8E-1
	channel regulator activity	3	3.2	5.1E-2	4.4E-1
	protein C-terminus binding	4	4.3	5.6E-2	4.6E-1
	lipid binding	7	7.5	5.9E-2	4.6E-1
	sodium:dicarboxylate symporter activity	2	2.2	6.0E-2	4.5E-1
	molecular adaptor activity	3	3.2	6.4E-2	4.7E-1
	calcium ion transmembrane transporter activity	2	2.2	7.2E-2	4.9E-1
	ATPase activity, coupled to transmembrane movement of ions	3	3.2	7.6E-2	5.1E-1
	ATPase activity, coupled	5	5.4	8.7E-2	5.4E-1
	glucose transmembrane transporter activity	2	2.2	8.9E-2	5.4E-1
	sugar:hydrogen symporter activity	2	2.2	9.4E-2	5.5E-1
	cation:sugar symporter activity	2	2.2	9.4E-2	5.5E-1
	ion channel inhibitor activity	2	2.2	9.4E-2	5.5E-1
	dicarboxylic acid transmembrane transporter activity	2	2.2	9.4E-2	5.5E-1
	structural molecule activity	8	8.6	9.5E-2	5.5E-1
	channel inhibitor activity	2	2.2	1.0E-1	5.6E-1
	calcium channel regulator activity	2	2.2	1.0E-1	5.6E-1

Category	Term	Count	%	P-Value	Benjamini
GOTERM_CC_FAT	organelle inner membrane	15	16.1	8.3E-9	1.8E-6
	mitochondrial inner membrane	13	14.0	2.6E-7	2.8E-5
	organelle membrane	22	23.7	1.2E-6	8.8E-5
	mitochondrial membrane	13	14.0	3.7E-6	2.0E-4
	mitochondrial envelope	13	14.0	7.0E-6	3.0E-4
	organelle envelope	15	16.1	1.7E-5	6.1E-4
	envelope	15	16.1	1.8E-5	5.4E-4
	membrane-bounded vesicle	14	15.1	3.1E-5	8.2E-4
	neuron projection	11	11.8	3.7E-5	8.7E-4
	respiratory chain	6	6.5	8.6E-5	1.8E-3
	cytoplasmic membrane-bounded vesicle	13	14.0	1.0E-4	2.0E-3
	NADH dehydrogenase complex	5	5.4	1.1E-4	2.0E-3
	respiratory chain complex I	5	5.4	1.1E-4	2.0E-3
	mitochondrial respiratory chain complex I	5	5.4	1.1E-4	2.0E-3
	vesicle	14	15.1	1.7E-4	2.7E-3
	actin cytoskeleton	9	9.7	2.1E-4	3.2E-3
	mitochondrial part	13	14.0	2.1E-4	3.0E-3
	vesicle membrane	7	7.5	2.9E-4	3.9E-3
	cytoskeleton	20	21.5	4.2E-4	5.3E-3
	cytoplasmic vesicle	13	14.0	4.3E-4	5.0E-3
	mitochondrial respiratory chain	5	5.4	5.9E-4	6.6E-3
	mitochondrial membrane part	6	6.5	9.3E-4	9.9E-3
	synapse	9	9.7	1.3E-3	1.3E-2
	cytoplasmic vesicle membrane	6	6.5	1.5E-3	1.4E-2
	synapse part	7	7.5	3.6E-3	3.3E-2
	clathrin coated vesicle membrane	4	4.3	4.0E-3	3.5E-2
	cytoplasmic vesicle part	6	6.5	5.4E-3	4.5E-2
	endocytic vesicle	4	4.3	5.4E-3	4.3E-2
	nucleosome	4	4.3	6.4E-3	5.0E-2
	synaptic vesicle membrane	3	3.2	8.3E-3	6.2E-2
	cell projection	11	11.8	8.8E-3	6.3E-2
	coated vesicle membrane	4	4.3	9.7E-3	6.7E-2
	mitochondrion	14	15.1	1.2E-2	8.3E-2
	protein-DNA complex	4	4.3	1.5E-2	9.6E-2
	axon	5	5.4	1.5E-2	9.6E-2
	intracellular non-membrane-bounded organelle	25	26.9	1.6E-2	9.6E-2
	non-membrane-bounded organelle	25	26.9	1.6E-2	9.6E-2
	pigment granule	4	4.3	1.6E-2	9.7E-2
	melanosome	4	4.3	1.6E-2	9.7E-2
	dendrite	5	5.4	1.7E-2	9.6E-2
	membrane fraction	11	11.8	2.3E-2	1.3E-1
	insoluble fraction	11	11.8	2.8E-2	1.5E-1
	calcineurin complex	2	2.2	3.0E-2	1.5E-1
	spectrin-associated cytoskeleton	2	2.2	3.0E-2	1.5E-1
	contractile fiber part	4	4.3	3.1E-2	1.5E-1
	trans-Golgi network	3	3.2	3.1E-2	1.5E-1
	plasma membrane part	21	22.6	3.4E-2	1.6E-1
	contractile fiber	4	4.3	3.6E-2	1.7E-1
	growth cone	3	3.2	4.2E-2	1.9E-1
	site of polarized growth	3	3.2	4.3E-2	1.9E-1
	clathrin-coated vesicle	4	4.3	4.5E-2	1.9E-1
	spliceosome	4	4.3	4.5E-2	1.9E-1
	spectrin	2	2.2	5.3E-2	2.2E-1
	cytosol	14	15.1	5.3E-2	2.2E-1
	membrane raft	4	4.3	5.5E-2	2.2E-1
	cell cortex	4	4.3	5.8E-2	2.3E-1
	cytoskeletal part	11	11.8	5.9E-2	2.2E-1
	cell fraction	12	12.9	5.9E-2	2.2E-1
	postsynaptic density	3	3.2	6.8E-2	2.5E-1
	coated vesicle	4	4.3	7.1E-2	2.5E-1
	synaptic vesicle	3	3.2	7.7E-2	2.7E-1
	plasma membrane	30	32.3	7.7E-2	2.6E-1
	cell junction	7	7.5	9.2E-2	3.0E-1
	synaptosome	3	3.2	9.3E-2	3.0E-1
	secretory granule	4	4.3	9.5E-2	3.0E-1
	endomembrane system	9	9.7	9.8E-2	3.1E-1
	heterogeneous nuclear ribonucleoprotein complex	2	2.2	9.8E-2	3.0E-1